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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Sherie L. Morrison, et al.
Serial No.	:	To Be Assigned
Filed	:	(concurrently herewith as a continuation of application Serial no. 07/893,610 filed June 3, 1992)
For	:	RECEPTORS BY DNA SPLICING AND EXPRESSION
Group Art Unit	:	To Be Assigned
Examiner	:	To Be Assigned

June 27, 1994

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Applicants have filed a continuation of application serial no. 07/893,610 concurrently herewith and now request amendment of the application.

IN THE CLAIMS

Applicants respectfully request entry of the following amendments to the pending claims.

39. (Four times amended) A method for producing a functional antibody comprising [having] a heavy chain and a light chain, which comprises the steps of:

(a) transfecting a non-antibody producing mammalian cell with a first DNA sequence coding for a first chain of the antibody;

(b) transfecting the cell with a second DNA sequence, said second DNA sequence coding for a second chain of the antibody, said second chain being a chain other than

the first chain and said first and second chains being either the heavy chain or the light chain; and

(c) maintaining the cell in a nutrient medium, so that the cell expresses the first and second DNA sequences and the resultant chains are intracellularly assembled together to form the antibody which is then secreted in a form capable of specifically binding to antigen.

43. (Twice amended) A method as recited in claim 78 [39] wherein the cell is a myeloma cell.

54. (Thrice amended) A method for producing a functional antibody comprising [having] a heavy chain and a light chain, which comprises the steps of:

(a) transfecting a non-antibody producing mammalian cell with a plasmid comprising a first DNA sequence coding for a first chain of the antibody and a second DNA sequence coding for a second chain of the antibody, said second chain being a chain other than the first chain and said first and second chains being either the heavy chain or the light chain; and

(b) maintaining the cell in a nutrient medium so that the cell expresses said first DNA sequence and said second DNA sequence and the resultant chains are intracellularly assembled together to form the antibody which is then secreted in a form capable of specifically binding to antigen.

55. (Amended) A method as recited in claim 39 wherein the antibody is a chimeric antibody comprising [having] a variable region substantially the same as that

found in a first mammalian source and comprising [having] a constant region substantially the same as that found in a second mammalian source, said second mammalian source being from a mammalian species other than that of the first mammalian source.

60. (Amended) A method as recited in claim 84 [54] wherein the cell is a myeloma cell.

66. (Amended) A method as recited in claim 54 wherein the antibody is a chimeric antibody comprising [having] a variable region substantially the same as that found in a first mammalian source and comprising [having] a constant region substantially the same as that found in a second mammalian source, said second mammalian source being from a mammalian species other than that of the first mammalian source.

67. (Amended) A method for producing a functional antibody comprising [having] a heavy chain and a light chain which comprises the steps of:

(a) maintaining in a nutrient medium a non-antibody producing mammalian cell, said cell having been transfected with a first DNA sequence coding for a first chain of the antibody and a second DNA sequence coding for a second chain of the antibody, said second chain being a chain other than the first chain and said first and second chains being either the heavy chain or the light chain;

(b) expressing from said cell the heavy chain and the light chain functionally assembled together to form said

antibody which is then secreted in a form capable of binding antigen; and

(c) recovering said antibody.

71. (Amended) A method as recited in claim 90 [67] wherein the cell is a myeloma cell.

72. (Amended) A method as recited in claim 90 [71] wherein the cell is a murine myeloma cell.

77. (Amended) A method as recited in claim 67 wherein the antibody is a chimeric antibody comprising [having] a variable region substantially the same as that found in a first mammalian source and comprising [having] a constant region substantially the same as that found in a second mammalian source, said second mammalian source being from a mammalian species other than that of the first mammalian source.

Please add the following claims:

78. A method for producing a functional antibody comprising a heavy chain and a light chain, which comprises the steps of:

(a) transfecting a non-antibody producing lymphoid cell with a first DNA sequence coding for a first chain of the antibody;

(b) transfecting the cell with a second DNA sequence, said second DNA sequence coding for a second chain of the antibody, said second chain being a chain other than the first chain and said first and second chains being either the heavy chain or the light chain; and

(c) maintaining the cell in a nutrient medium, so that the cell expresses the first and second DNA sequences and the resultant chains are intracellularly assembled together to form the antibody which is then secreted in a form capable of specifically binding to antigen.

79. A method as recited in claim 78 wherein the cell is transfected via protoplast fusion.

80. A method as recited in claim 78 wherein the cell is transfected via calcium phosphate precipitation.

81. A method as recited in claim 78 wherein the cell does not endogenously produce any immunoglobulin chains.

82. A method as recited in claim 78 wherein the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain but not both.

83. A method as recited in claim 78 wherein the antibody is a chimeric antibody having a variable region substantially the same as that found in a first mammalian source and having a constant region substantially the same as that found in a second mammalian source, said second mammalian source being from a mammalian species other than that of the first mammalian source.

84. A method for producing a functional antibody comprising a heavy chain and a light chain, which comprises the steps of:

(a) transfecting a non-antibody producing lymphoid cell with a plasmid comprising a first DNA sequence coding for a first chain of the antibody and a second DNA sequence coding for a second chain of the antibody, said second chain being a chain other than the first chain and said first and second chains being either the heavy chain or the light chain; and

(b) maintaining the cell in a nutrient medium so that the cell expresses said first DNA sequence and said second DNA sequence and the resultant chains are intracellularly assembled together to form the antibody which is then secreted in a form capable of specifically binding to antigen.

85. A method as recited in claim 84 wherein the cell is transfected via protoplast fusion.

86. A method as recited in claim 84 wherein the cell is transfected via calcium phosphate precipitation.

87. A method as recited in claim 84 wherein the cell does not endogenously produce any immunoglobulin chains.

88. A method as recited in claim 84 wherein the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain but not both.

89. A method as recited in claim 84 wherein the antibody is a chimeric antibody comprising a variable region substantially the same as that found in a first mammalian

source and comprising a constant region substantially the same as that found in a second mammalian source, said second mammalian source being from a mammalian species other than that of the first mammalian source.

90. A method for producing a functional antibody comprising a heavy chain and a light chain which comprises the steps of:

(a) maintaining in a nutrient medium a non-antibody producing lymphoid cell, said cell having been transfected with a first DNA sequence coding for a first chain of the antibody and a second DNA sequence coding for a second chain of the antibody, said second chain being a chain other than the first chain and said first and second chains being either the heavy chain or the light chain;

(b) expressing from said cell the heavy chain and the light chain functionally assembled together to form said antibody which is then secreted in a form capable of binding antigen; and

(c) recovering said antibody.

91. A method as recited in claim 90 wherein the cell is transfected via protoplast fusion.

92. A method as recited in claim 90 wherein the cell is transfected via calcium phosphate precipitation.

93. A method as recited in claim 90 wherein the cell does not endogenously produce any immunoglobulin chains.

94. A method as recited in claim 90 wherein the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain but not both.

95. A method as recited in claim 90 wherein the antibody is a chimeric antibody comprising a variable region substantially the same as that found in a first mammalian source and having a constant region substantially the same as that found in a second mammalian source, said second mammalian source being from a mammalian species other than that of the first mammalian source.

Please cancel claims 52, 53, 56, 59 and 70.

already cancelled

REMARKS

In the parent application, application serial no. 07/893,610, agreement was reached with the Examiner with respect to the allowability of the pending claims as indicated in the December 10, 1993 Examiner Interview Summary Record, paper no. 31. The allowability of those claims was withdrawn in the subsequent December 27, 1993 final office action, paper no. 32.

Applicants have filed a continuation application herewith and submit this Preliminary Amendment to limit the issues for appeal. The following remarks are responsive to the December 27, 1993 action.

This application claims a method for producing a functional antibody and is related to previously pending application Serial No. 07/771,410 which claimed functional antibodies produced by the method of the instant application. In Paper no. 15 of the '410 application, an Advisory Action dated May 12, 1993, the Examiner noted that "[a]pplicants' arguments may be persuasive with respect to method, but not for product ..."* Applicants submit that the record of the '410 application, including the arguments that applicants have presented therein, supports the patentability of the pending method claims.

Enablement

In paragraph 17 of the Office Action, the Examiner has rejected the pending claims as enabling only the expression of "a chimeric polypeptide which is a subunit of an immunoglobulin molecule", for reasons stated in papers 5,

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* The claims in the related application were rejected on legal grounds regarding patentability requirements specific to product-by-process claims.

7 and 10. That reasoning found that claims drawn to a method for producing a "receptor" were too broad and could include the production of Major Histocompatibility Complex antigens and T-cell receptors. However, applicants have cancelled claims 49-51 that contained that broader language. That cancellation obviates this rejection. The remaining claims cover a method for producing a "functional antibody". Functional antibodies are disclosed as the product of the method described in the present application and are fully supported by the specification.

The Examiner has also made an enablement rejection based on information in the Rule 131 Declaration of Dr. Morrison. In response to the Examiner's invitation in the Office Action, applicants submit the following explanation to show enablement of the instant invention in non-antibody producing mammalian cells.

The Examiner's conclusion that the invention is not enabled for any cell lines other than the particular cell line used is based on the erroneous premise that only 1 in 4 of the experiments performed is operative to show antibody binding. The January 22, 1992 declaration of Dr. Morrison submitted previously to show diligent reduction to practice sets forth the work done after transforming the J558L cells. In paragraph 10 of that declaration Dr. Morrison described the performance of SDS gel electrophoresis on the expression product of the transfected J558L cells. That gel confirmed that applicants had successfully obtained fully assembled antibody molecules.

Paragraph 11 of that declaration describes Dr. Morrison's attempt to show that those antibodies were capable of specific binding, but the substance that she was

using to radio-label the antibodies was of poor quality. Therefore that assay was unsuccessful due to chemical problems with the analytical tool, not because of any problem with the expression product.

When Dr. Morrison retested the antibody with a new preparation of the radio-label, the ELISA assay successfully showed binding activity as described in paragraph 12 of her declaration. Thus, applicants successfully showed binding the first time they used an appropriate test. An ELISA test is what Cabilly (A and R) relied on to show antigen binding. However, applicants believed that a more stringent and reliable test for binding should subsequently be performed using a PC column.

Dr. Morrison's first attempt to show binding with a PC column was not successful as described in Paragraph 13 of the declaration. To investigate this, Dr. Morrison ran another ELISA assay and discovered that the transfected cell line had likely lost the ability to express the transfected kappa light chain gene. The loss of transfected gene expression often occurs and Dr. Morrison reacted by re-transfecting the kappa light chain gene as described in paragraph 15 of her declaration. After re-transfecting the exogenous light chain gene, the analysis of the expression product with the PC column described in paragraph 15 suggested specific binding.

In order to obtain a more certain result, Dr. Morrison changed the selection marker on the light chain vector and re-transfected the cells with that new vector as described in paragraph 18 of her declaration. The suggested mutation mentioned in paragraph 18 is the same mutation mentioned in paragraph 14, namely that the cell line had

lost its ability to produce the transfected kappa light chain gene. In Paper No. 26 the Examiner stated that "the J558L cell line underwent some unexplained mutation ... which resulted [in] the single chain loss mutant becoming a double chain loss mutant." However, the mutation to which Dr. Morrison referred in her declaration was the loss of expression of the exogenous kappa light chain gene, not the loss of expression of the endogenous lambda light chain gene*. Because there was no suspicion of a mutation in the endogenous gene and because antigen binding by the expression product of the transfected genes was shown via ELISA both before and after the re-transfection of the kappa light chain gene, the expression of antibodies was not a peculiarity of any mutation in the cell line with which Dr. Morrison was working.

As the Examiner points out, the phosphate buffer, not the loss of the transfected kappa chain gene, may have been the reason no binding was obtained in the first run with the PC column. Elimination of that buffer as described in paragraph 18 removed an impediment to specific antigen binding and allowed Dr. Morrison to demonstrate again that she had obtained antibodies capable of specific binding.

Because applicants successfully showed antigen binding with the PC column and with ELISA both before and after the suspected loss of expression of the transfected kappa chain, it is not the case that only 1 of 4 experiments is operative in the instant claims. Applicants submit that

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* The J558L cell line did not become a double chain loss mutant. To make certain that the record is clear, applicants define a non-antibody producing cell as one that does not express, secrete and assemble functional antibodies. Such a cell is not necessarily a double chain loss mutant; the cell may express and secrete a single immunoglobulin chain.

the repeated positive results rebut the basis for asserting a lack of enablement of other non-producing cell lines.

There is nothing in the present record that shows this invention cannot be practiced in any mammalian cell. The record shows that Dr. Morrison achieved repeated positive results in lymphoid cells and that Dr. Morrison believed that those results were predictive of success in other mammalian cells. (August 18, 1993 § 1.132 Declaration of Dr. Morrison). The Examiner has pointed to no mammalian cell in which the claimed method is inoperative.

The Examiner points out that CHO and COS cells are different than lymphoid cells, but does not assert that the present invention is not operative in those cell lines. In fact, co-transfection of heavy and light antibody chain genes in CHO and COS cells does result in expression of functional antibodies as shown in articles published after the filing date of applicants' parent application (see e.g., Weidle et al., Gene vol. 51, pp. 21-29 (1987); Feys et al., Int. J. Cancer, supp. 2, pp. 26-27 (1988)). Accordingly, there is no basis for a § 112 rejection for lack of enablement because there is no evidence of inoperativeness. The evidence shows that co-transfection and co-expression works in various mammalian cell lines.

Applicants note that the present amendment adds claims to producing antibodies in lymphoid cells. Those claims comply with the Examiner's position that "[a]pplicant's claims should be limited to lymphoid cell lines" and, therefore, should not be subject to a § 112 rejection.

Obviousness

The pending claims stand rejected under 35 U.S.C. § 103 as obvious over Cabilly (L, R or 2A) or Boss (2B) in view of Gillies(S). Applicants respectfully traverse this rejection.

The Examiner suggests that Cabilly's teaching of producing two immunoglobulin chains by expressing two exogenous genes in bacteria, and Cabilly's mention of mammalian host cells, taken together with Gillies' production of a single exogenous chain that assembles with endogenous chains to form a tetramer, renders applicants' invention obvious. However, at the time of the invention many additional circumstances precluded one who would try to combine these teachings from having a reasonable certainty that they would succeed in producing functional antibodies.

A basis of the obviousness rejection is that mammalian systems were known to functionally express exogenous immunoglobulin genes. Applicants agree that the prior art did teach transfecting and expressing a single exogenous gene coding for one chain of an immunoglobulin in a mammalian cell which expresses an endogenous gene coding for another immunoglobulin chain. Gillies et al., Cell, 33, pp. 717-28 (1983); Oi et al., Proc. Natl. Acad. Sci. USA, 80, pp. 825-29 (1983); Rice et al., Proc. Natl. Acad. Sci. USA, 79, pp. 7862-65 (1983). However, the endogenous-exogenous approach followed in each of these references produced tetrameres that were not functional antibodies because the binding sites of the exogenous immunoglobulin chains were not complimentary to the binding sites of the endogenous chains. Indeed, prior to applicants' invention,

no exogenous immunoglobulin gene had been functionally expressed.

Further, prior to the present invention, it was not even believed that the endogenous-exogenous approach would always produce an immunoglobulin, let alone one that binds an antigen. In Oi, a mouse myeloma cell supported expression of a transfected immunoglobulin gene. However, a rat myeloma cell line which expresses and secretes an endogenous light chain, when treated in the same manner as the mouse myeloma, did not support expression of a transfected immunoglobulin chain gene.

In sum, prior to the present invention, the art as a whole taught that a non-producing cell would not necessarily express a transfected exogenous immunoglobulin chain gene. Thus, applicants certainly would not have had a reasonable expectation that such a cell would successfully express, secrete and assemble the product of that first exogenous gene with the product of a second exogenous gene. Consequently, applicants' invention is not obvious in view of the prior art.

Also in paragraph 18, the Examiner states that the instant claims cannot be given the August 1984 priority date. On that date the first application in the chain that led to the present application was filed. It disclosed the production of functional antibodies by co-transfecting a mammalian cell with exogenous DNA. In August 1987 a CIP application (Serial No. 07/090,669) was filed in which material was added to the specification to specifically address the production of intra-species chimeric antibodies. Additional claims were filed based on that new matter.

However, on March 22, 1991, the new claims to intra-species chimeras were cancelled. The new material added to the specification in 1987 is not relied on in any way to support the instant claims. The new disclosure in the specification did not narrow the invention such that applicants are now trying to avoid it; the new disclosure was additive and applicants are no longer seeking claims based on the new matter.

Because the instant claims are fully supported by material originally disclosed in the parent application, they are entitled to the benefit of the August 1984 filing date. Accordingly, for reasons of record in previously filed papers, Boss(S) is not prior art to applicants' invention.

In response to the Examiner's invitation in paragraph 18, and to show unexpected results -- a secondary consideration in an obviousness determination --, applicants previously submitted the August 18, 1993 Rule 132 Declaration of Sherie L. Morrison. The Federal Circuit has explained that

> "[O]bjective evidence such as commercial success, failure of others, long-felt need, and unexpected results must be considered before a conclusion on obviousness is reached. Id. (citing Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed.Cir. 1986), cert. denied, 480 U.S. 947, 107 S.Ct. 1606, 94 L.Ed.2d 792 (1987)). Indeed, as then Chief Judge Markey said in Stratoflex, Inc. v. Aeroquip Corp., 713 F.2d 1530, 1538, 218 USPQ 871, 879 (Fed.Cir. 1983), 'evidence of secondary considerations may often be the most probative and cogent evidence in the record. It may often establish that an invention appearing to have been obvious in light of the prior art is not.'" Minnesota Min. and Mfg. v. Johnson & Johnson, 976 F.2d 1559, 1573, 24 USPQ2d 1321, 1333 (Fed.Cir. 1992).

Dr. Morrison's declaration explains laboratory data that establish an unexpectedly high yield of functional antibody.

Applicants' initial analysis of the expression product of their co-transformed cells was performed without attention to maximizing yield, but rather merely to demonstrate specific binding. Nonetheless, that analysis demonstrated an unexpectedly high 32% yield of active, assembled antibody. (Morrison Declaration, ¶ 5, submitted herewith.) In fact, that yield is likely a significant underestimate of the amount of correctly assembled antibody produced. (Morrison Declaration, ¶ 8.)

Cabilly's denaturation/renaturation approach to "recombination" of light and heavy chains resulted in an extremely low level of antibody formation -- only 0.76% of the chains are reported as having "recombined" to form antibody. Applicants achieved a yield that is over 42 times greater than the amount of active antibody that Cabilly reported and, therefore, is unexpectedly high when compared with Cabilly's .76% yield.

One might expect an improved yield when expressing a mammalian gene in a mammalian environment as applicants did. Even expecting to double the yield might be reasonable. However, applicants have obtained a wholly unexpected improvement in yield that is 42 times the yield cited in the Cabilly patent. This result is of both statistical and practical significance. (Morrison Declaration, ¶¶ 9-10.)

Even Cabilly's .76% yield may be an overstatement. Cabilly's 0.76% yield is based on an estimate of the levels of heavy and light immunoglobulin chains in the reaction mixtures and on an antigen binding assay (Cabilly A,

col. 27, lines 11-16). Not only is this calculated yield dubious by virtue of its reliance on an estimate with unknown associated error, but the calculation also used as a background number the binding measured for cells producing light chains only. A more accurate background number to correct for non-specific binding would have been the antigen binding for cells producing only heavy chains because it has been found that heavy chains alone will bind antigen in the absence of a complementary light chain. Ward et al., Nature, vol. 341, pp. 544-46 (1989). That number would likely have been much higher than the light chain background number and would have resulted in a lower percent yield. Even given this potential for overestimating percent recombination, Cabilly calculated obtaining only a fraction of one percent of the antibody protein in active form.

Whether or not Cabilly's yield is an accurate estimate, applicants' yield is so much larger as to be an unexpected result. Such results constitute a secondary consideration weighing against a finding of obviousness.

The Examiner has invited applicants to clarify the record as to why the unexpected yield of a single cell line would be predictive of all cell lines. As set forth in Dr. Morrison's declaration (¶¶ 11-13), co-expression of exogenous genes in J558L, a cell that did not differ from other mammalian cells in its ability to express endogenous genes, would be predictive of similar co-expression of exogenous genes in those other mammalian cells. While applicants have discussed the original unpredictability of whether it would work at all to produce functional antibodies by co-expression, applicants have not stated any reason that once co-expression was found to work, other

mammalian cells that are similarly transfected would behave differently. Indeed, the Rule 132 Declaration of Dr. Morrison states reasons to believe that other mammalian cells would behave similarly.

The non-obviousness of applicants' approach is also evidenced by the reaction of those skilled in the art. In 1984, Michael Boss congratulated one of the present inventors for, in essence, beating him to the production of functional antibodies by using eukaryotic cells instead of bacteria. (Morrison Declaration, ¶ 14.) Such praise for the invention is another secondary consideration that supports a finding of nonobviousness. Interconnect Planning Corp. v. Feil, 774 F.2d 1132, 1143-44 (Fed.Cir. 1985).

Applicants continue to assert that their invention is non-obvious without need to rely on secondary considerations. The examiner states that "[g]iven Cabilly's explicit suggestion of mammals, one of ordinary skill in the art would have had ample motivation to combine the teachings of the Cabilly reference with the attendant advantages known to result from mammalian cell expression."

However, Cabilly's statements about co-expression follow:

> "When heavy and light chain are coexpressed in the same host, the isolation procedure is designed so as to recover reconstituted antibody. This can be accomplished in vitro as described below, or might be possible in vivo in a microorganism which secretes the IgG chains out of the reducing environment of the cytoplasm." ((emphasis added) Cabilly A, col. 13, lines 19-25.)

Cabilly only asserts the possibility that co-expression would work in microorganisms and provides absolutely no suggestion that co-expression "might be possible" in

mammalian cells. Clearly, there is no suggestion here that co-expression should be combined with the mammalian immunoglobulin expression found in Gillies.

Even accepting the Examiner's argument that the requirements for enablement under 35 U.S.C. § 112 are far more stringent than the requirements under 35 U.S.C. § 103,* there must be some suggestion to combine the references. Neither Cabilly nor Gillies suggests combination with the other.

The Examiner also states that the "Gillies [paper] provides a vast increase in yield with respect to the Cabilly patent." However, Gillies does not report any yield of functional antibodies. No functional antibodies were sought nor obtained. The yield reported by Dr. Morrison was the yield of antigen-binding antibodies. She did not include in that yield any single chains or non-binding tetramers that may have been expressed. Accordingly, the yield of correctly assembled and folded functional antibody was a surprising result even given the Gillies reference.

With respect to the August 19, 1993 Morrison § 1.132 declaration, the detailed basis showing why the statistical significance is "certain" is given in ¶¶ 8-9. Dr. Morrison states that her yield is a likely underestimate, that her yield is 42 times greater than Cabilly's yield, and that this increase in yield could not be within the range of experimental error.

In view of the foregoing, applicants believe that the pending claims are in condition for allowance.

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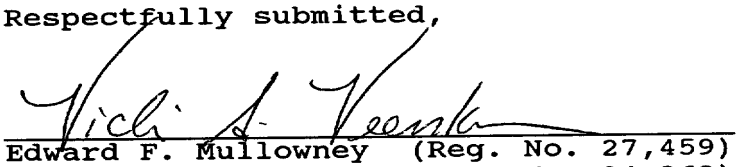
* The Examiner cites In re Lukach and Chester v. Miller for this proposition. However, both of those cases relate to differences in the requirements for adequate written descriptions versus anticipation. Neither addresses enablement versus obviousness.

Accordingly, entry of the present amendment and allowance of the claims are requested.

Respectfully submitted,



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